J. Helminthol. Soc. Wash.
60(2), 1993, pp. 183–204

Ultrastructure of the Infective-Stage Larva of *Toxocara canis* (Nematoda: Ascaridoidea)

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ABSTRACT: The ultrastructural morphology of the infective-stage larva of *Toxocara canis* is described. Seven weeks after eggs were placed in culture in 0.5% formalin, larvae were hatched mechanically and collected 2 days later. Larvae were fixed 3 days at 4°C in aldehyde fixative, postfixed in osmium tetroxide, embedded, sectioned, and stained. The cuticle has several layers of fibers, and lateral alae extend the length of the body. The lateral cord hypodermis has multiple nuclei, mitochondria, and lipid granules. Muscle cells are meromyarian and platymyarian. A neuronal bundle that innervates the cephalic sensillae runs anteriad from the nerve ring on each side of the worm. The ventral nerve cord has numerous nuclei, mitochondria, and neural fibers. The excretory cell has a single large nucleus, extensive rough endoplasmic reticulum (RER), Golgi bodies, mitochondria, and vesicles presumably containing protein; the 2 excretory columns also have vesicles surrounding a collecting duct. The dorsal sector of the esophagus is much larger than the 2 subventral sectors and contains RER, Golgi bodies, and vesicles with variable density suggesting a maturation of their content. The intestine has no lumen and is composed of a single row of cells containing lipid granules. The rectum is lined with cuticle.

KEY WORDS: *Toxocara canis,* larval morphology, ultrastructure, nematode.

Larval toxocariasis in humans is caused in most instances by the larvae of *Toxocara canis* (Beaver et al., 1984). In humans and other paratenic hosts, the larvae that persist in the tissues are morphologically the same as the larvae that hatch from infective eggs (Nichols, 1956a; Beaver et al., 1984). The morphology of these larvae has been described in detail at the level of the light microscope (Nichols, 1956a). Although other workers have examined various aspects of the ultrastructural morphology of these larvae (Rockey et al., 1983; Ghafoor et al., 1984; Vegni-Talluri et al., 1986; Vegni-Talluri and Dallai, 1990), there has been no overview of the fine structure of these larval nematodes presented. Thus, the purpose of this work was to provide a generalized description of the fine structure of these larval nematodes.

Materials and Methods

Infective-stage larvae from eggs that had been in culture for 2 mo were collected for in vitro cultures using the methods of Bowman et al. (1987). One day after the larvae were placed in culture, they were transferred to 1-ml centrifuge tubes. The tubes containing the larvae were centrifuged at 7,000 *g* for 1 min, and the larvae were resuspended in modified Karnovsky's fixative (1.25% glutaraldehyde and 20% paraformaldehyde in 0.1 M phosphate buffer, pH 7.0) and fixed at 4°C for 3 days. The aldehyde fixative was removed using an overnight wash of 0.1 M phosphate at a pH of 7.0; this and all the other solution changes made prior to the addition of agar as described below were done by centrifuging the larvae at 7,000 *g* for 1 min and suspension in new solution. The larvae were postfixed for 1 hr in 1% osmium tetroxide and then washed twice with 2 10-min changes of distilled water. After removing the water from the second wash, a small portion of 2% agar, about 150 μl, at 55–60°C was added to the pellet of larvae. After the agar hardened, it was removed from the tubes and cut into small blocks. Blocks of larvae were dehydrated using a graded ethanol series and then infiltrated with the plastic embedding mixture of Mollenhauer (1964). Infiltration was performed using mixtures of plastic resin in propylene oxide: the blocks were in the 25% resin mix for 1 hr, the 50% resin mix for 2 hr, the 75% resin mix for 3 hr, and the pure epoxy mix overnight. The infiltrated blocks of larvae were embedded in fresh plastic resin.

Sections were cut using a Reichert Ultracut E microtome and were either mounted on mesh grids or transferred using a formvar film suspended across a small wire hoop, to slot grids. Sections were stained with uranyl acetate, or uranyl acetate and lead citrate, and were examined using a Philips 410 electron microscope. Photographs were recorded on Kodak electron image plates.

Description (Figs. 1–25)

STOMA AND ESOPHAGUS: The stoma is composed of cuticle that is more electron translucent than that of the anterior end (Fig. 1). It is triradiate, and the external surface of the stoma is lined with cuticle that is of the same density as

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that of the surface of the worm (Figs. 1, 2). At the level of the vestibule, the internal surface of the cuticle, as described by Vegni-Talluri et al. (1986), is lined with numerous interdigitations of the lamellae composed of the plasma membranes of the vestibular cells (Fig. 1). The cuticle lining the lumen of the esophagus extends the length of this structure (Figs. 1–18). Esophageal cells appear to attach to the esophageal luminal cuticle via zonular junctions arranged parallel to the long axis of the esophagus (Figs. 3–16).

The esophagus is divided by the cuticle-lined, triradiate lumen into 3 sectors, 1 dorsal and 2 subventral, and there is no significant torsion away from the dorsoventral axis throughout the length of the esophagus (Figs. 3–16). The esophagus can be divided into 4 region on the basis of morphology: a slender procorpus (Figs. 3, 4), a thickened metacorpus (Figs. 5–7), a slender elongated isthmus (Figs. 8–14), and a terminal ventriculus (Figs. 15–17). Posterior to the excretory pore, the esophagus is apparently pushed into the dorsal portion of the body by the large excretory cell (Figs. 10–16).

The esophagus contains 3 gland cells; there is 1 gland cell in each sector. The dorsal gland cell is the largest and most extensive; it extends from the beginning of the metacorpus to the esophageal valve (Figs. 5–17). At the level of the ventriculus, the enlarged dorsal gland cell causes the dorsal sector to be several times larger than the subventral esophageal sectors. The nucleus of the dorsal gland cell is quite large and is located at the very posterior portion of the ventriculus (Fig. 17). The subventral esophageal gland cells are located almost exclusively within the ventriculus extending only slightly anteriad into the isthmus (Fig. 13). The nuclei of the subventral gland cells are also located within the ventriculus at a level slightly anteriad to that of the dorsal gland cell (Fig. 17). The cytoplasm of the dorsal and 2 subventral gland cells are similar; the cytoplasm contains mitochondria, rough endoplasmic reticulum (RER), Golgi lamellae, and vesicles of varying densities. The collecting cisternae described by Vegni-Talluri et al. (1986) were not seen in these sections.

INTESTINE AND PROCTODEUM: The intestine consists of a single chain of cells, each with 1 large nucleus (Figs. 18–25). Also observed within the intestinal cells are numerous, smaller inclusions that have an appearance similar to small nuclei (Fig. 25). Near the esophagus, the intestinal cells are laterally compressed by the excretory columns (Fig. 18), but more posteriorly, the excretory columns are smaller and the intestinal cells become more circular in cross section (Figs. 19–21). The cytoplasm of the intestinal cells contains large lipid granules and scattered deposits of glycogen.

The proctodeum is lined with cuticle that is similar to that lining the lumen of the esophagus (Fig. 24). This cuticle appears continuous with the surface of the worm. This cuticle-lined channel extends between the anus and the last intestinal cell and is surrounded by numerous nuclei with very small amounts of associated cytoplasm (Figs. 22, 24).

CUTICLE: The cuticle is composed of 4 layers that are designated herein as the epicuticle, cortical, fibrillar (medial), and matrix (basal) layers (Fig. 26). The cuticle is about 0.4 μm thick at midbody. Striae are present from near the anterior end of the worm (Fig. 1) to the very tip of the tail (Fig. 24); the striae are about 0.8 μm apart. The outermost layer of the cuticle is the epicuticle; it is a thin, electron-dense layer that covers the entire external surface of the cortical layer. Progressing internally, the next layer is the cortical layer, and running throughout this layer is the slightly more electron-dense fibrillar layer. The fibrillar layer is present near the external layer of the cortical layer and forms thickenings at the base of each stria (Fig. 17). The matrix layer extends the length of the worm under the cortical layer as a flat, homogeneous layer that has the same thickness throughout the body.

Lateral alae begin slightly posteriad to the buccal capsule (Fig. 4), become very prominent at the level of the nerve ring (Fig. 9), and extend posteriad past the rectum to near the tip of the tail (Figs. 22, 23). At the base of each ala, the dorsal and ventral cortex is modified by the addition of an electron-opaque, V-shaped layer that divides the less dense cortex (e.g., Fig. 12); this more dense layer is evident from the most anterior extent of the ala (Fig. 4) to its most posterior extent (Fig. 23). The V-shaped layer is external to the unchanged matrix and fibrillar layers of the cuticle that are also present in each ala (Fig. 27). Alae are about 3 μm tall at midbody (Fig. 18).

HYPODERMIS: At a level just posterior to the buccal capsule, the hypodermis of the lateral cords is composed of cytoplasm containing numerous mitochondria (Figs. 3, 4). This same cytoplasm is contiguous with that between the muscle cells and cuticle in the 4 body quadrants, and at this level it forms the areas of the dorsal and ventral cords (Figs. 3, 4). Slightly posterior to this level,

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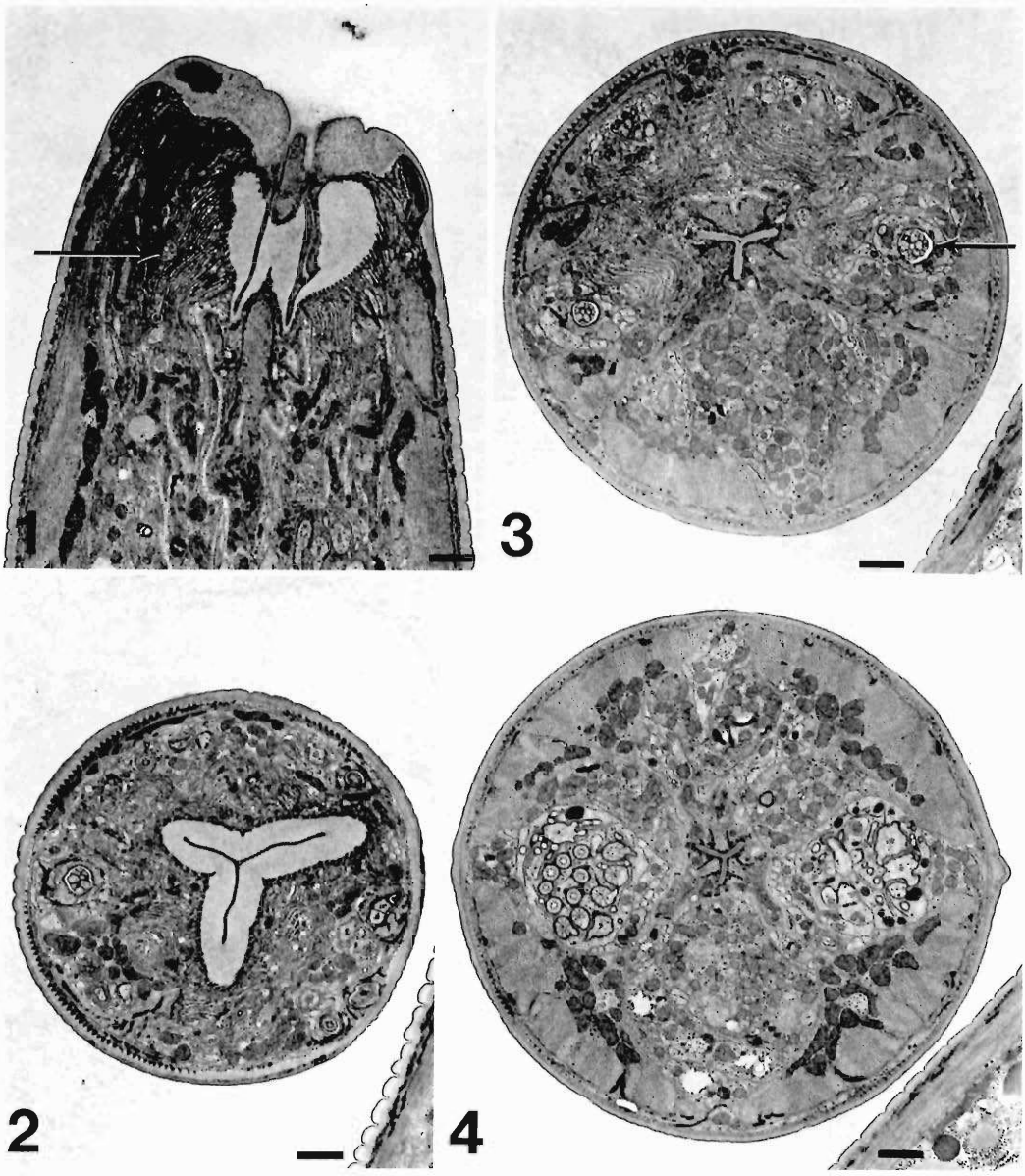

Figures 1–4. Electron micrographs of the infective-stage larva of *Toxocara canis*. Bars = 1 μm. 1. Anterior end, lateral view. Note the prolonged ventral surface and the translucent vestibule attached to the lamellae of the vestibular cells (arrow). 2. Transverse section at the level of the vestibule. Note the various prolongations of the anterior sensory neurons. 3. Transverse section just posterior to the vestibule; again note the anterior projections of the sensory neurons (arrow at 1 amphid) and the thick fibrillar areas attached to the cuticular lining of the esophagus. 4. Transverse section at the level of the esophageal procorpus. Note the large bundles of sensory neurons located laterally on the worm, the presence of lateral alae with inner cuticular bars, the large numbers of mitochondria in the lateral cords, and the small number (2) of muscle cells per quadrant.

the lateral cord hypodermis has a small central area next to the cuticle that is separated from its adjacent areas by desmosomes (Fig. 5). These desmosomes are present throughout the length of the worm, being present even posterior to the anus (Figs. 5–13, 15, 16, 18–23). This medial area of hypodermis extends into the pseudocoelom between the sublateral portions of the cord

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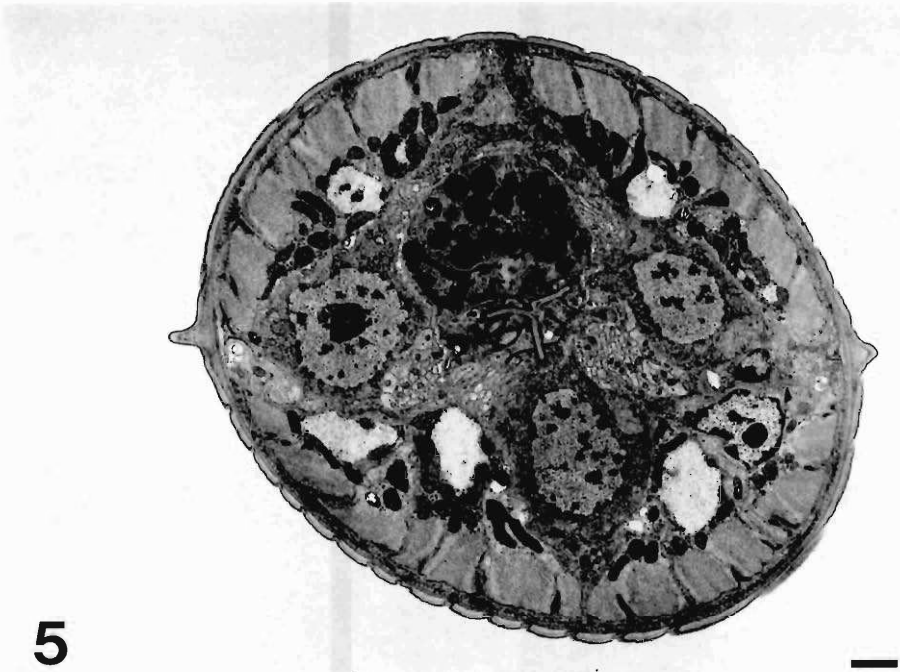

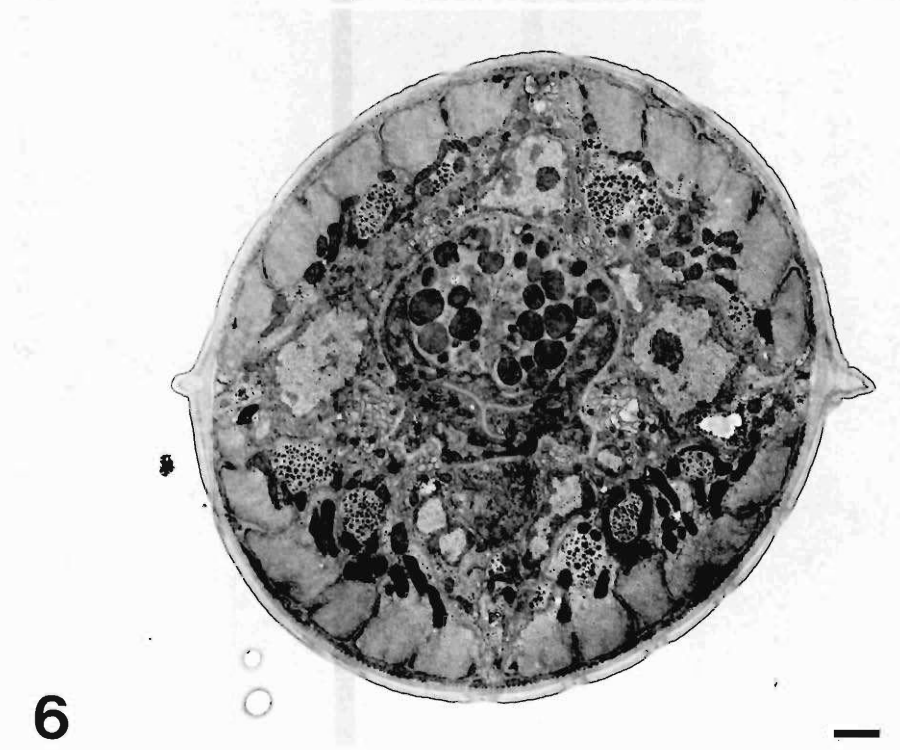

Figures 5, 6. Electron micrographs of the infective-stage larva of *Toxocara canis*. Bars = 1 μm. 5. Transverse sections at the level of the metacorpus. Note the enlargement of the dorsal sector of the esophagus, the presence of a centrally demarcated area within the lateral cord, and the large cell nuclei within the pseudocoelom. 6. Transverse section slightly posteriad to Figure 5. Note the ventral and dorsal cords.

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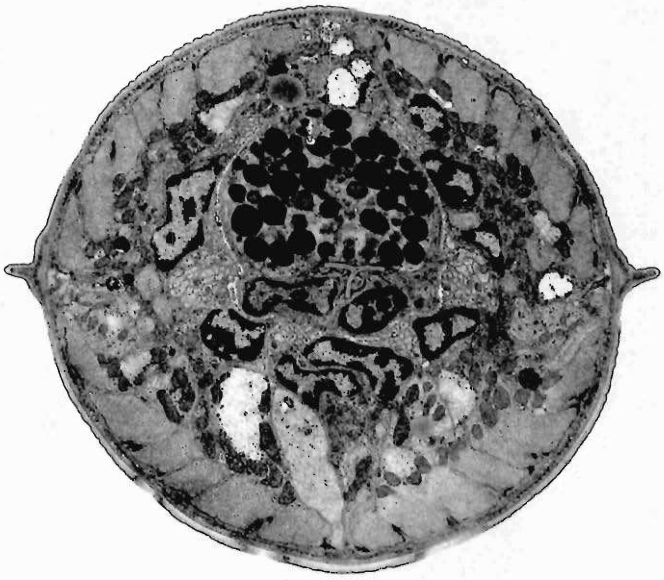

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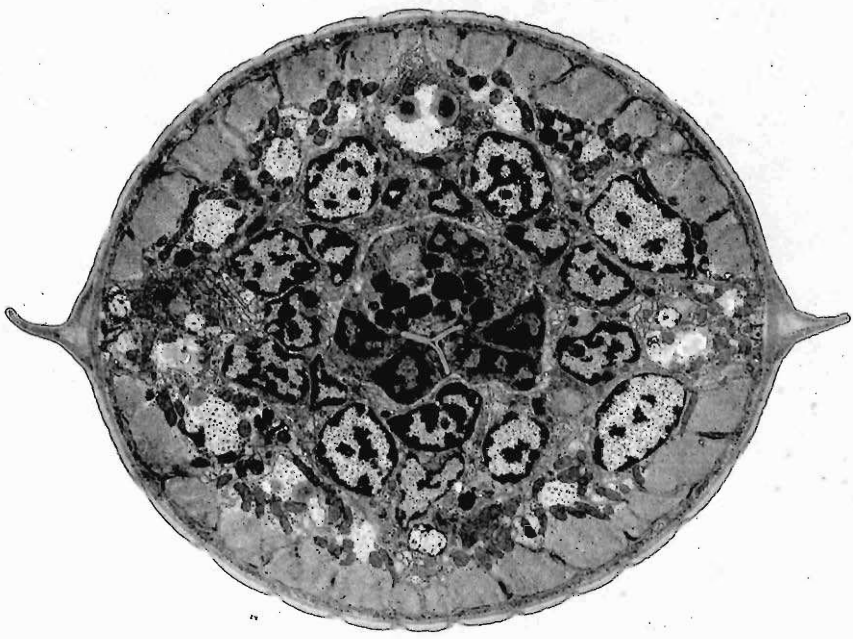

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Figures 7, 8. Electron micrographs of the infective-stage larva of *Toxocara canis*. Bars = 1 μm. 7. Transverse section at the level of the metacorpus. Note the appearance of numerous nerve bundles and nuclei within the pseudocoelom. 8. Transverse section just anterior to the nerve ring and posterior to the esophageal metacorpus. Note the large number of nuclei within the pseudocoelom, the well-demarcated areas within the lateral cords, and the large number of nerve fibers that are present.

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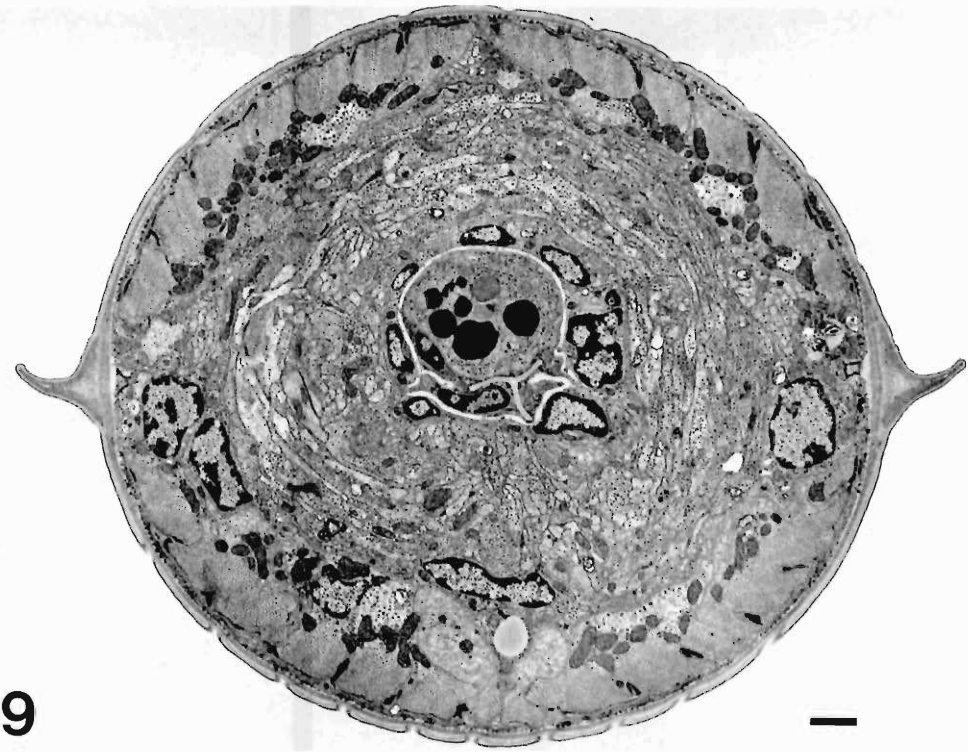

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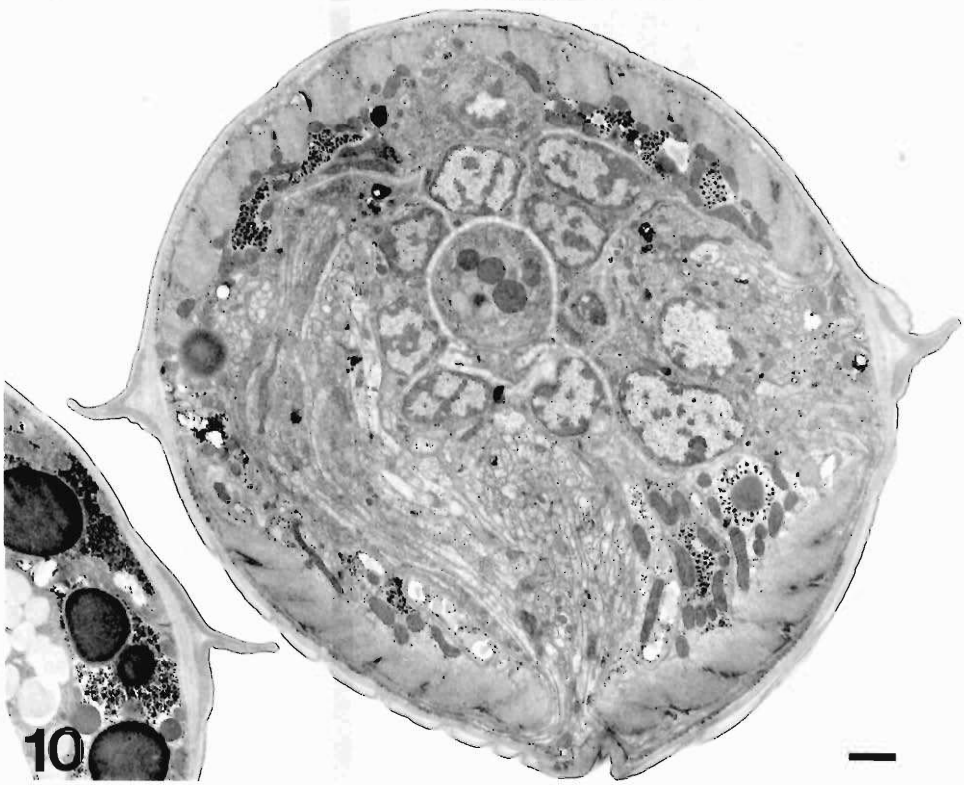

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(Figs. 12, 16, 20, 28). At the level of the deirid (Fig. 11), this same area of cytoplasm extends into the subcuticular papillary prominence. Mitochondria were sometimes observed in this portion of the hypodermis (Figs. 8, 11, 12, 15, 16, 20), but nuclei were observed only at the level of the rectum (Fig. 22). There were no similar regions demarcated by desmosomes in the dorsal and ventral cords, although the hypodermis extends into the pseudocoelom in these areas (Figs. 15, 16). The sublateral portions of the lateral cords are apparent at the level of the nerve ring as granular cytoplasm containing nuclei and numerous mitochondria (Figs. 8, 9). At the level of the excretory cell nucleus (Fig. 12), these portions of the cord are quite prominent and contain mitochondria, nuclei, and large lipid droplets; this morphology is consistent throughout the remainder of the body of the worm (Figs. 13–16, 18–24). Posterior to the anteriormost occurrence of desmosomes in the lateral cords, the sublateral portions of the lateral cord are the portions of hypodermis that appear contiguous with that lying between the cuticle and muscle cells. In the hypodermis under the muscle cells of the body quadrants, numerous tonofilamentlike densities extend between the cuticle and the underlying muscle cells; these densities are not present under the lateral alae (e.g., Fig. 12).

SOMATIC MUSCULATURE: The somatic musculature begins just posterior to the buccal capsule (Figs. 1, 3) and extends to near the tip of the tail (Fig. 25). The muscle cells are meromyarian and platymyarian in type. Anteriorly, there are 2 cells per quadrant (Fig. 3); at the nerve ring through the base of the esophagus, 3 cells per quadrant (Figs. 9–13, 15, 16); at the beginning of the intestine, 3 cells per quadrant (Fig. 18); in the region of the posterior intestine, 2 cells per quadrant (Fig. 19); and posterior to the anus, 1 cell per quadrant (Figs. 22, 23). There are usually several bundles of myofibrils per muscle cell (e.g., Fig. 16). The cytoplasmic portions of the muscle cells contain numerous mitochondria and glycogen (e.g., Figs. 11, 15) as well as the muscle cell nucleus (e.g., Fig. 16).

NERVOUS SYSTEM: The nerve ring is a circular bundle of fibers surrounding the esophagus (Fig. 9). Although a few nuclei are present in the nerve ring, most nuclei are confined to areas just anterior to and just posterior to the nerve ring (Figs. 8 and 10, respectively). Within the fibers of the nerve ring are numerous small vesicles and mitochondria (Fig. 9).

The ventral cord is the major nerve trunk running throughout the worm. In the area of the stoma, the cytoplasm of the cells in the cord contain Golgi apparati, numerous mitochondria, and multivesicular bodies (Figs. 2–4). Posterior to this level, but anterior to the nerve ring, the cord typically contains a single nucleus or a bundle of nuclei surrounded by an area of mitochondria and RER (Figs. 5–7). In the anterior nucleated portion and the nonnucleated portion of the nerve ring, it is similar to the other nervous tissue (Figs. 8, 9). At the level of the excretory pore, numerous fibers from the nerve ring extend into the ventral cord (Fig. 10); more posteriad, neuronal fibers and nuclei surround the serpentine excretory duct (Fig. 11). From the excretory cell commissure to the tail, the ventral cord contains neurons and cell bodies with nuclei, mitochondria, and neuronal fibers (Figs. 18, 23). Posterior to the rectum, the ventral cord fills most of the pseudocoelom (Fig. 23). The dorsal nerve cord is similar to the ventral cord throughout its length but is smaller in diameter.

The sensory structures of the anterior end are innervated by fascicles of fibers extending anteriad from the nerve ring (Figs. 2–8); the largest fascicles are laterally located (Figs. 4–8). At the anterior of the lateral alae, some of the nerves in the lateral fascicles contain microtubules in the pattern of a modified ciliary axoneme, a circle of 9 doublet microtubules and an inner group of 2–6 single microtubules (Fig. 4). There are at least 13 of these tubule-bearing cells at the base of each amphidial socket (Fig. 4), but not all extend to the anterior extremity of the worm (Figs. 2, 3). The nerves that innervate the outer labial pupillae also have a similar, microtubular

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Figures 9, 10. Electron micrographs of the infective-stage larva of *Toxocara canis*. Bars = 1 μm. 9. Transverse section at the level of the nerve ring. Note the lack of nuclei within the nerve ring that fills the pseudocoelom. Also note the highly expanded lateral alae. 10. Transverse section at the level of the excretory pore. Note the large numbers of nerve fibers extending into the ventral cord at this level and the appearance of more neural nuclei within the pseudocoelom.

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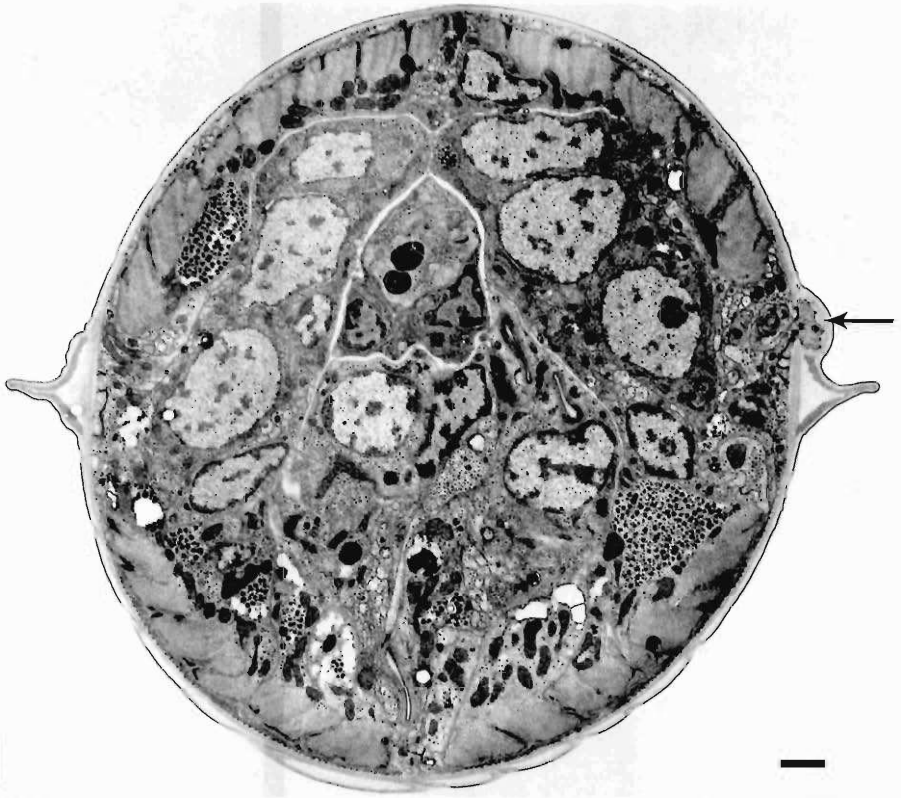

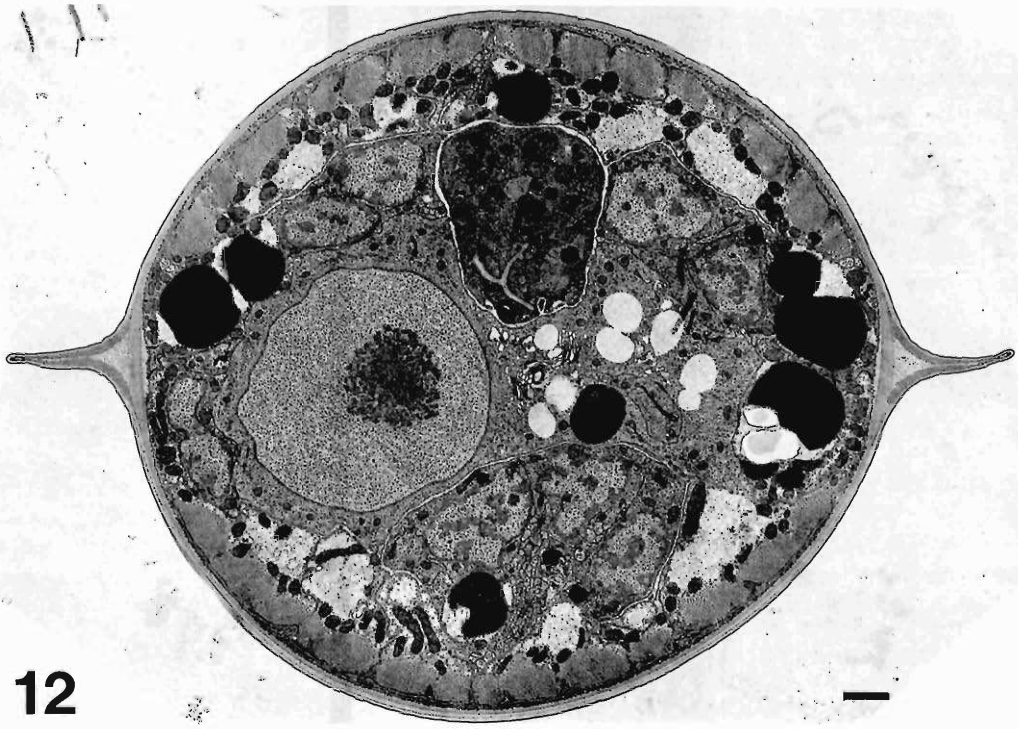

Figures 11, 12. Electron micrographs of the infective-stage larva of *Toxocara canis*. Bars = 1 μm. 11. Transverse section at the level of the left deirid (arrow). Note the extension of nervous tissue through the cuticle dorsal to the lateral ala. Sinuous tracts of the excretory duct can also be seen. 12. Transverse section at the level of the excretory cell commissure. Note the large excretory cell nucleus and the numerous Golgi bodies within the excretory cell cytoplasm. The lateral alae are probably most pronounced at this level.

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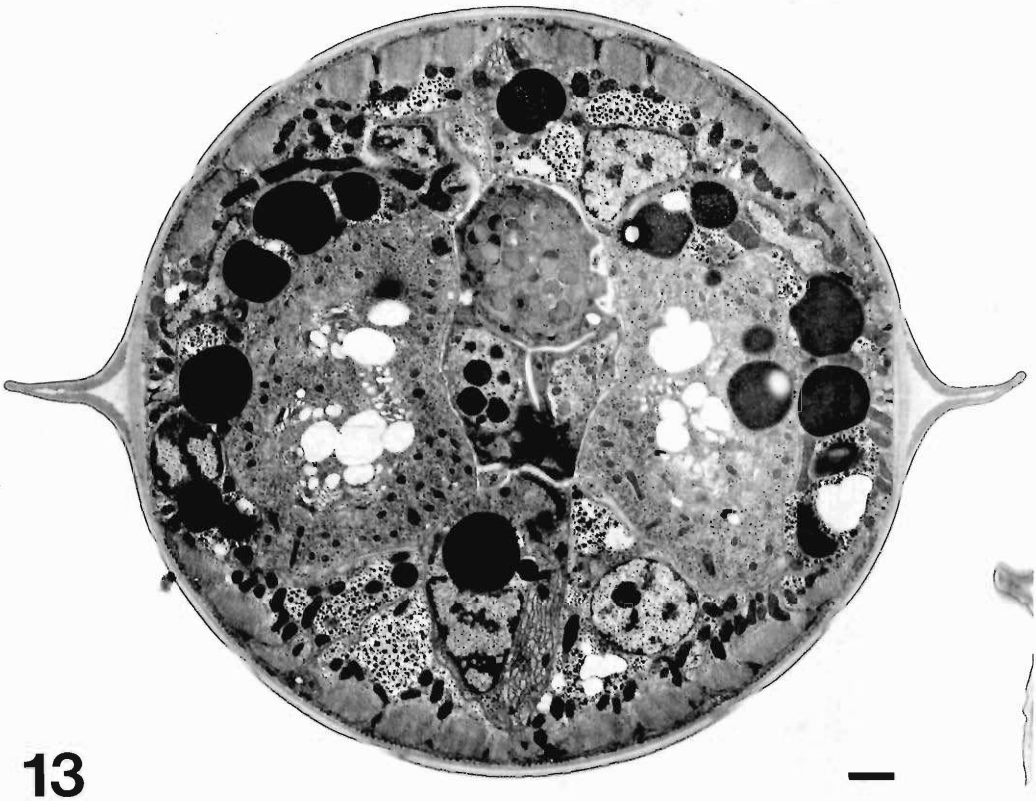

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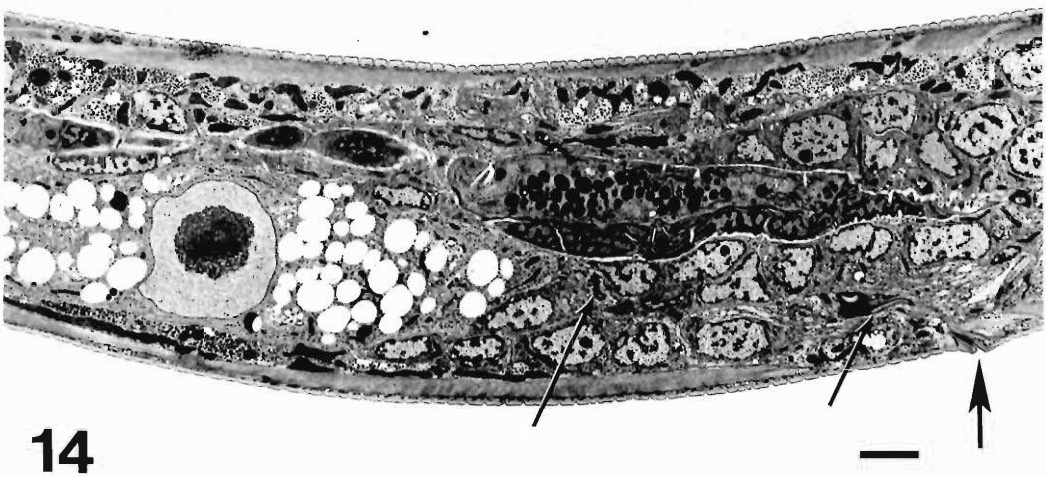

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Figures 13, 14. Electron micrographs of the infective-stage larva of *Toxocara canis*. **13.** Transverse section just posterior to the excretory cell commissure. Note the beginning of the posteriorly directed excretory columns, the large ventral ganglion, and the anterior extension of the subventral gland cells of the esophagus in their respective subventral sector. Bar = 1 μm. **14.** Longitudinal section through the anterior end of the worm from the level of the excretory cell nucleus to the excretory pore. From the excretory pore (large arrow) the excretory duct continues posteriad (small arrows) to the large portion of the excretory cell where the large excretory cell nucleus is located. Bar = 2 μm.

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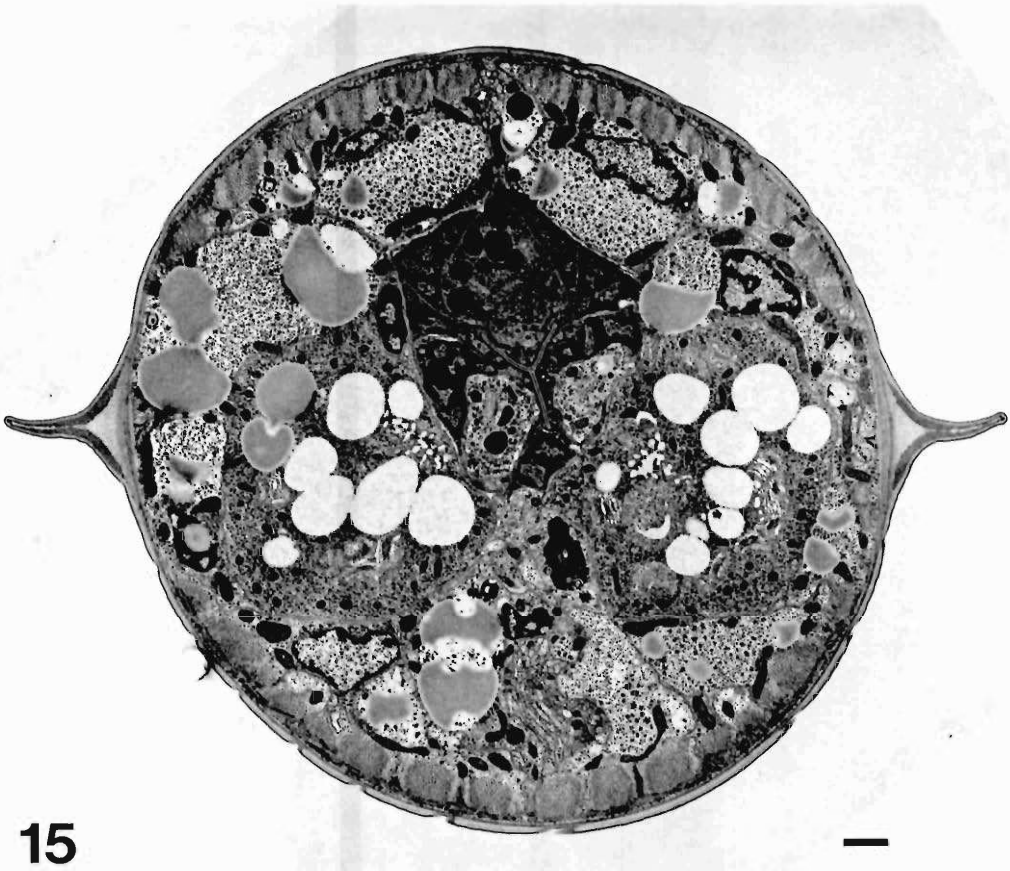

Figure 15. Transverse section just anterior to the ventriculus of the esophagus of the infective-stage larva of *Toxocara canis*. Note the large number of nuclei composing the esophagus and the presence of dorsal and subventral gland cell cytoplasm in the 3 esophageal sectors. Bar = 1 μm.

pattern (Fig. 2); the nerves of the inner labial papillae were not seen.

Deirids were located just posteriad to the excretory pore and just dorsal to the lateral alae. Each deirid was formed by a subcuticular protrusion of hypodermis and nervous tissue through the matrix and fibrillar layers of the cuticle (Fig. 11). Phasmids were not seen in sections.

EXCRETORY SYSTEM: The unicellular excretory system has the appearance of a shortened "H" as described by Nichols (1956a). The large nucleus (Figs. 12, 14) occurs at the level of the excretory cell commissure, i.e., where the anteriorly and posteriorly directed lateral columns join. From the excretory pore (Fig. 10), the sinuous excretory duct extends posteriad to the cell commissure (Figs. 11, 12). The excretory duct is lined with an electron-dense material that appears similar to the epicuticle of the body (Fig. 14).

The cytoplasm of the excretory cell contains numerous mitochondria, Golgi bodies, RER, and large numbers of vesicles that presumably contain protein. The vesicles are found throughout the excretory cell cytoplasm (Figs. 12–20), except in the lateral arms that extend anteriad from the

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Figures 16, 17. Electron micrographs of the infective-stage larva of *Toxocara canis*. Bars = 1 μm. 16. Transverse section through the ventriculus at the level of the left subventral gland nucleus. Note the dorsal displacement of the esophagus and the large subventral gland cell nucleus. The dorsal sector of the esophagus is enlarged and

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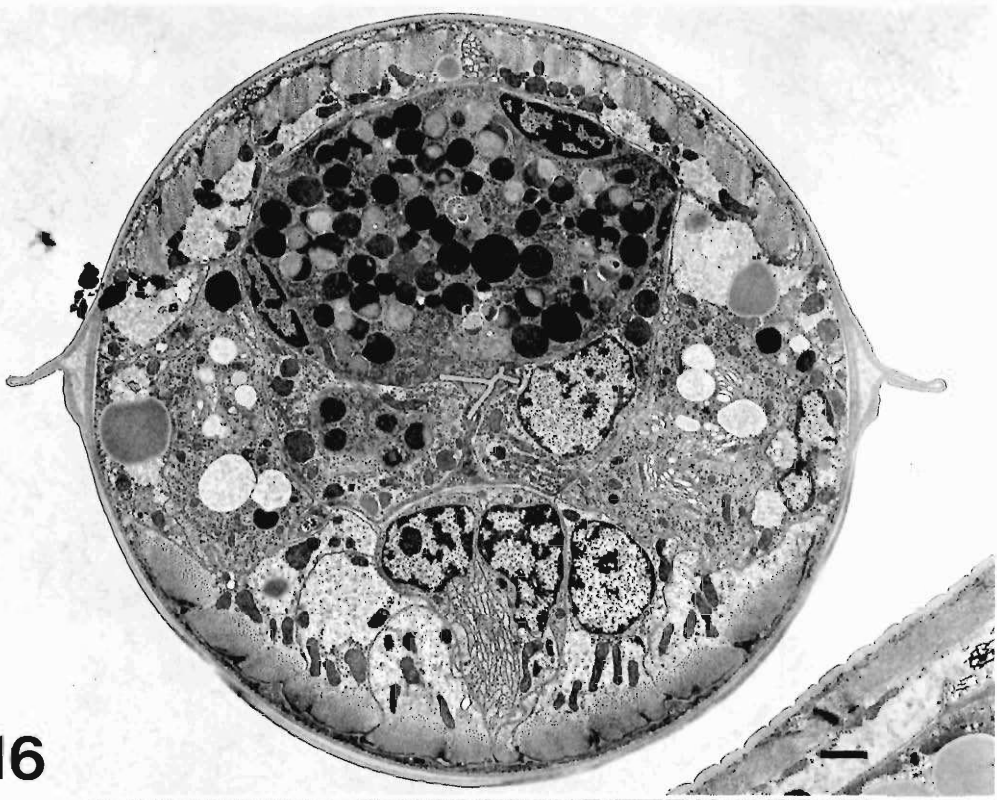

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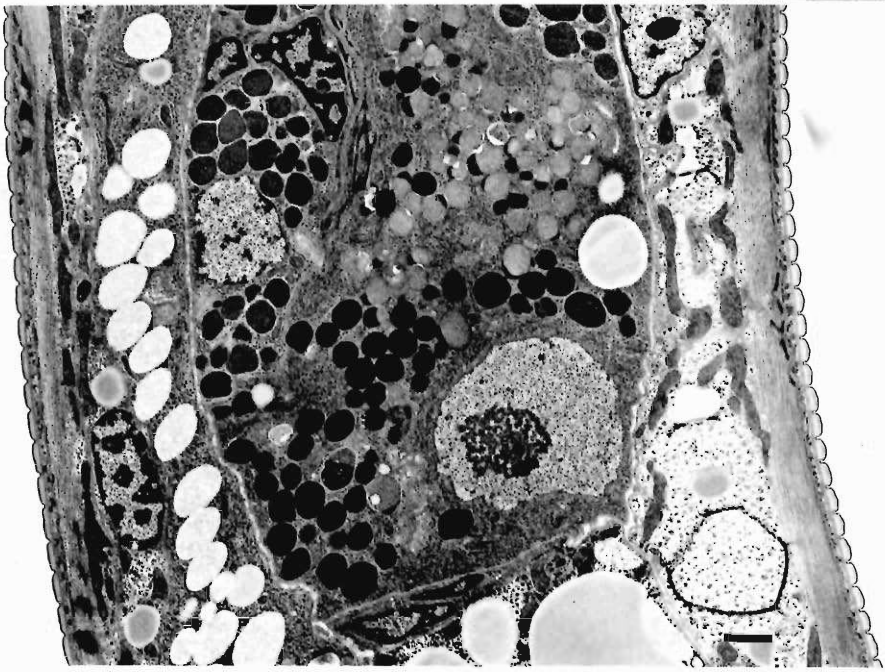

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filled with the dorsal esophageal gland. 17. Longitudinal section through the ventriculus of the esophagus. This section shows the relationship of the dorsal and subventral gland cell nuclei with respect to each other and with respect to the beginning of the intestine.

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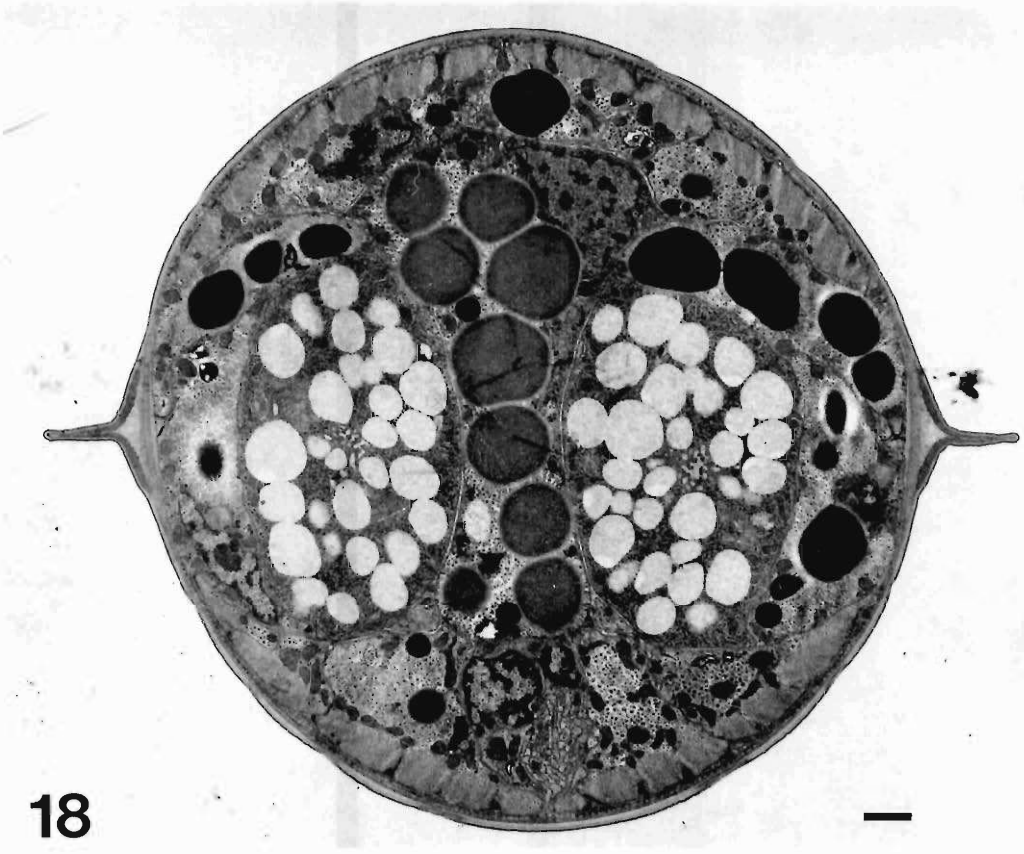

Figure 18. Transverse section at the level of midbody of the infective-stage larva of *Toxocara canis* showing the compression of the intestinal cell by the excretory cell processes. Also note the large ventral cord and the thickened cuticular bars within the lateral alae. Bar = 1 μm.

cell commissure. In the lateral columns posterior to the nucleus, the vesicles surround the collecting canaliculi. These canaliculi may join in the commissure to form the excretory duct, but this was not noted in any of the sections that were observed. The lateral columns extend further posteriad on the left side of the worm than on its right (Fig. 20).

GENITAL PRIMORDIUM AND PSEUDOCOELOMOCYTES: Although all sections in the region of the intestine were examined for these structures, cells were never identified that could be considered part of the genital primordium or that morphologically resembled pseudocoelomocytes.

Discussion

The present description extends the observations of Nichols (1956a) to the ultrastructural level. Overall, the description that was made with the light microscope was found to be very complete and is only supplemented by details.

The stoma of the infective stage larva of *T. canis* has been described previously (Vegni-Talluri et al., 1986). Those authors first reported on the lamellar system surrounding the vestibular cuticle and suggested that the resemblance of this lamellar system to the transporting epithelia of other animals indicates a possible function in ionic or osmotic regulation. They speculated that this osmotic regulation might be important as the larva encounters different environments within the infected host. All osmotic shifts would occur via diffusion through the overlying layer of thickened cuticle. The stomas of other infective-stage larvae of ascaridoid nematodes have not to our knowledge been examined for a similar type of structure. Such a structure is lacking in

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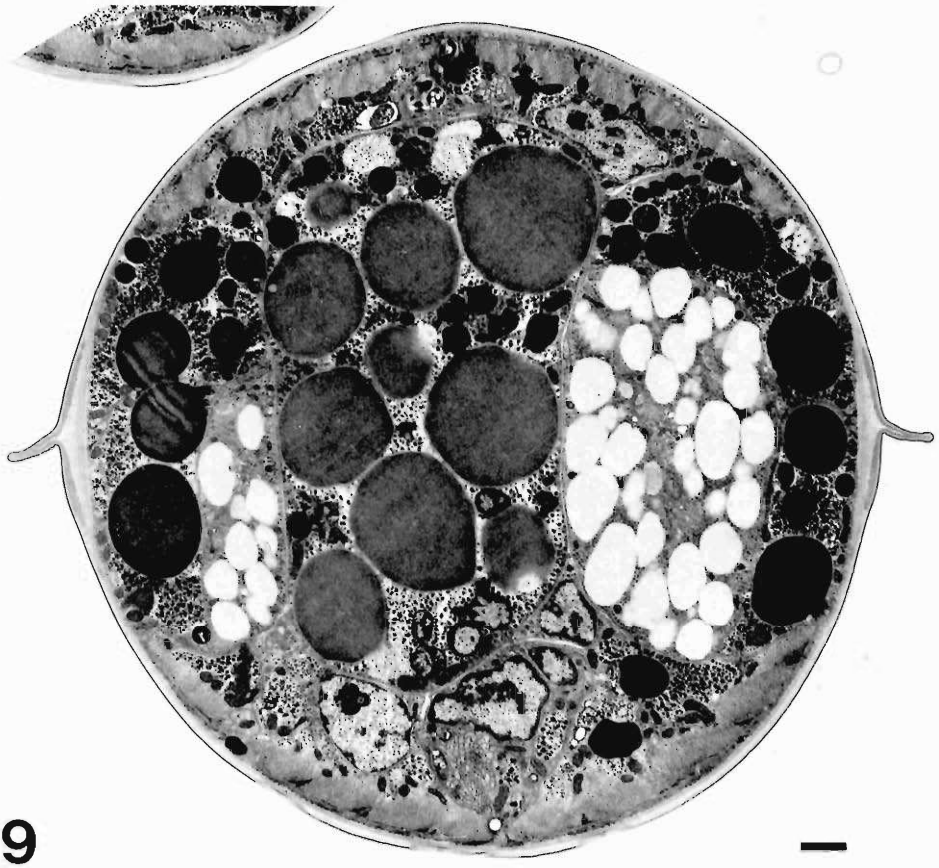

Figure 19. Transverse section of the infective-stage larva of *Toxocara canis* showing the diminution of the excretory cell toward the posterior of the body. Note that the process of the excretory cell on the worm's left side is larger than that on the right and that the sublateral portions of the lateral cords are prominent and contain large numbers of lipid droplets. At this level, the intestine is no longer compressed by the excretory cell columns. Bar = 1 μm.

the buccal capsules of fourth-stage larvae of *Caenorhabditis elegans* (Wright and Thomson, 1981) and second-stage larvae of *Meloidogyne incognita* (Endo and Wergin, 1988).

There was no indication of the presence of a cephalic septum at the front of the esophagus, as has been described for adult ascaridoids by Inglis (1964); this could be considered as further evidence that the lips that develop in the fourth-stage larvae and the adult nematodes are due to a process of elongation of the cells of the lips, clavate cells, and lobus impar, rather than to the inward growth of the hypodermis. Studies on the morphology of the cephalic structures in developing larvae would be required to resolve the details of the origin of the structures.

The ultrastructure of the esophagus of the infective-stage larva of *T. canis* has been examined by Vegni-Talluri et al. (1986), who described the duct emptying the dorsal esophageal gland cell as occurring near the anterior portion of the esophagus. The subventral gland cells are noted by Vegni-Talluri et al. (1986) to empty into the esophageal lumen near the base of the esophagus. The anatomy of the esophagus in the larval *T. canis* is very similar to that reported by Hsü (1933) for the adult worm. Hsü found that the ducts of the gland cells emptied in the same areas of the esophagus in the adult as they do in the larva, as described by Vegni-Talluri et al. (1986). Hsü reported that within the adult, the nucleus of the dorsal esophageal gland was in the ventral portion of the ventriculus and that the subventral gland cell nuclei were found in dorsal positions.

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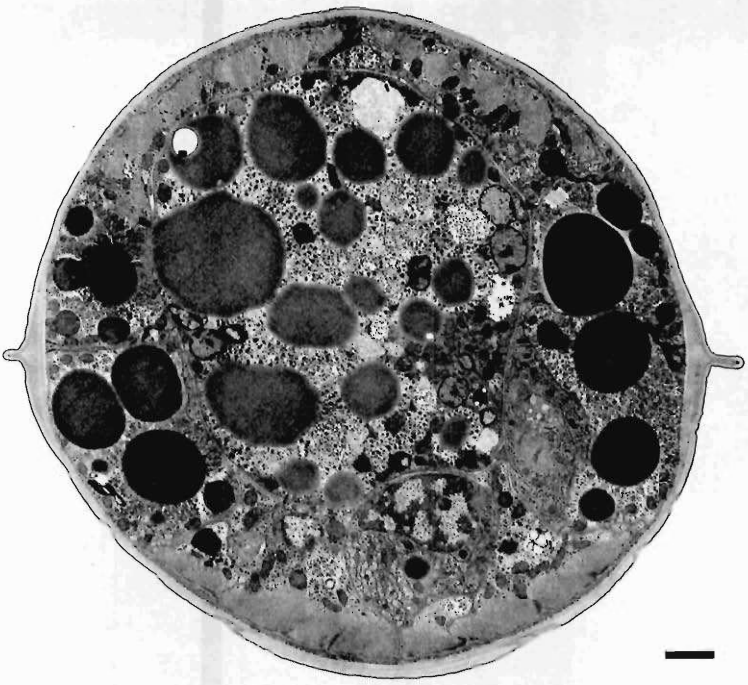

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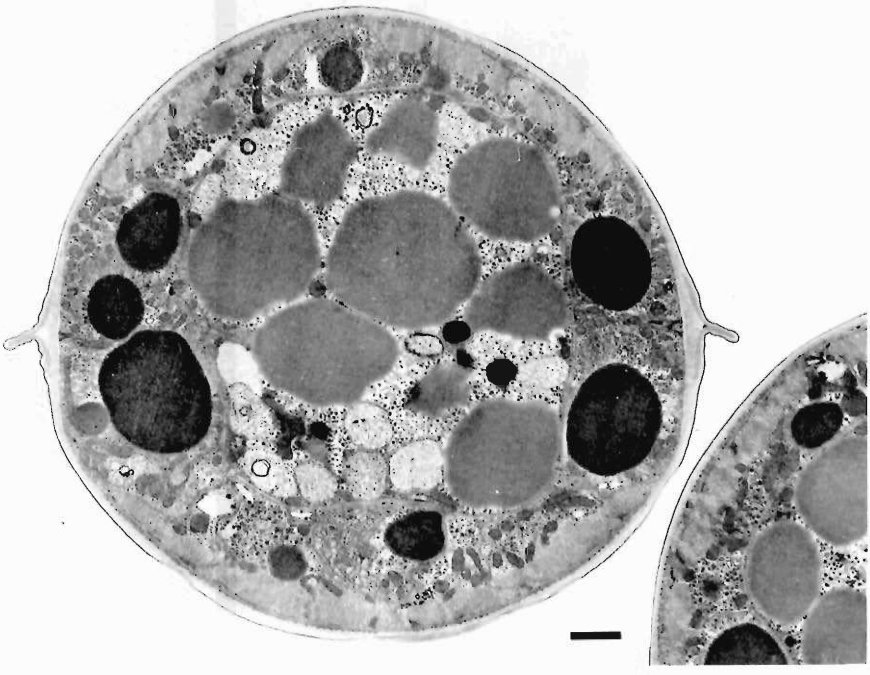

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Figures 20, 21. Electron micrographs of the infective-stage larva of *Toxocara canis*. Bars = 1 μm. 20. Transverse section near the end of the left posteriormost extension of the excretory column. Note that there is no excretory cell process on the worm's right side. The medial and sublateral portions of the lateral cord are quite prominent in this section. 21. Transverse section posterior to the excretory columns. The lateral cords at this level contain large numbers of mitochondria, and lipid droplets are present.

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The movement of the dorsal esophageal gland nucleus to a ventral position was noted in developing larvae that Sprent (1958) recovered from experimentally infected dogs. Schacher (1957), alternatively, noted the dorsal esophageal gland within the dorsal sector of the esophagus in fourth-stage larvae recovered from dogs but found that it had migrated to the ventral sector within the adult worms. Overall, however, it would appear that sometime during the development of the worm that these nuclei migrate to different positions within the ventriculus.

The granules within the esophageal glands of ascaridoids have received attention since they were first described by Looss (1896). Mueller (1931) examined the vesicles within the esophageal glands of the adult *Ascaris lumbricoides* and *Ascaris megacephala* and based on their fixation and staining reactions was convinced that they contained protein. The work of Drum (1966) on the secretory granules of the esophagus of adult *T. canis* and *A. lumbricoides* showed that they were membrane-bound vesicles containing endopeptidases with optimal activity similar to that of chymotrypsin. Work with larvae of the ascaridoid *Anisakis simplex* has also shown that the esophageal glands contain a trypsinlike proteolytic enzyme that was not present in the excretory cell (Matthews, 1984). Recent work with *Ostertagia circumcincta* (McGillivery et al., 1990) has shown that a stage-specific glycoprotein recovered from larval worms is located within the secretory vesicles of the esophagus. Similarly, work with the second-stage larva of *Meloidogyne incognita* has shown by immunogold labeling that a large molecular weight, secreted glycoprotein is located in the secretory granules of the subventral esophageal glands (Hussey and Mims, 1990; Hussey et al., 1990). The function of these glycoproteins has yet to be determined.

The intestine of the infective-stage larva of *T. canis,* as originally described by Nichols (1956a), is composed of a chain of single cells. The lack of a lumen has also been verified by Bai et al. (1978). In the canine final host, the cells of the intestine begin to multiply soon after infection. Schacher (1957) noted an intestinal lumen in larvae recovered from the stomach of dogs 3 days after infection with infective eggs. Similarly, Sprent (1958) noted larvae with intestinal lumina in worms recovered from naturally, prenatally, infected puppies during the first week of life. Griesemer et al. (1963) show a figure of larva of *T. canis* with a patent lumen in the lung of a puppy that was 2 days old. The intestines of the fourth-stage and adult worms are polycytous, as is typical of ascaridoid nematodes (Argeseanu, 1934; Chitwood and Chitwood, 1950; Fujino and Ishii, 1988).

The ultrastructure of the larval intestine of *T. canis* is very similar to that reported for the intestine of the infective-stage larva of *Ascaris suum* (Jenkins and Erasmus, 1971; Rubin and Trelease, 1975). The major feature of the intestine of both these worms is the large amount of stored glycogen and lipid. It has been postulated that the larvae use the glycogen for supplying energy during their migration in the host (Fairbairn, 1970); however, the ability of larvae to survive in cultures where they are metabolically active for periods of over a year (de Savigny, 1975) would indicate that these products might also be used for periods of low level activity within the eggshell when temperatures are warm enough to allow metabolism by the larvae. It has also been noted that the intestine of the lung-stage larva of *Ascaris suum* contains large quantities of phosphorylcholine within its intestinal tract (Gutman and Mitchell, 1977). Examinations as to the localization of phosphorylcholine within the larva of *T. canis* have not been performed.

The cuticle of the larval *T. canis* was found to correspond with the cuticle of the third-stage larvae of *Ascaris lumbricoides* as described by Thust (1966, 1968). It was also found to be very similar to the third-stage cuticle of *Ascaris suum* as described by Rockey et al. (1983) and Thompson et al. (1977). Unlike the larva of *T. canis,* the third-stage larva of *A. lumbricoides* does not have prominent lateral alae in the esophageal region (Nichols, 1956a; Thust, 1968).

The structure of the cuticle on the body was found to be different from that of the adults of both *Toxocara cati* (as described by Glaue, 1910a, b; and Erlich, 1937) and *Toxocara canis* (as described by Inglis, 1964). These authors reported a thick fiber layer as occurring under the matrix layer. Erlich and Inglis also described a series of "punctation canals" composed of fibers running from the fibrillar layer through the matrix layer to layers of dense fibers in the supporting fiber layers that are external to a basal lamellar layer. Neither thickened fiber layers nor punctation canals were observed in the cuticle of the infective larva. The morphology of the cuticle of the larvae is such that it would appear that the matrix layer

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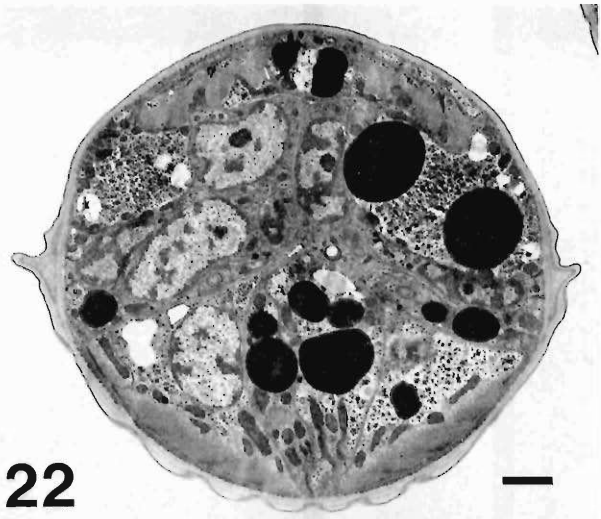

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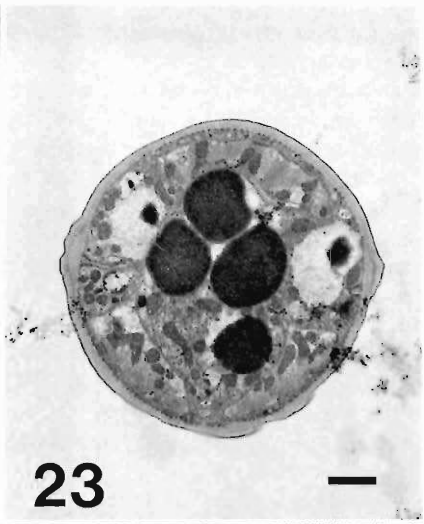

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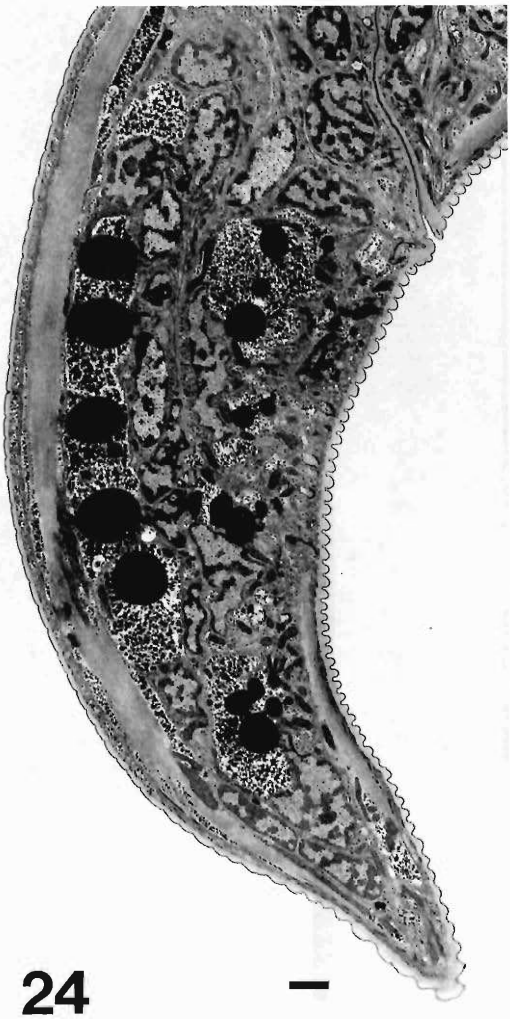

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is the innermost layer of the cuticle on the larva and that the other more internal layers do not develop either until the third-stage larva begins to grow or metamorphose to the fourth stage or perhaps even the adult stage.

The lateral alae of the infective-stage larva of *Toxocara canis* seen here were similar in morphology to those in the electron micrographs of Bai et al. (1978) and Kondo et al. (1987). Also, there were no differences seen in the alar morphology of the infective-stage larva when it was compared to the micrographs of lateral alae of larvae recovered from mice either 10 days (Bai et al., 1978) or sometime between 6 and 56 days (Ghafoor et al., 1984) after infection. A photomicrograph of a midbody section of an advanced larval stage in the lungs of a 2-day-old puppy shows alae that are different from those of the infective-stage larva in that they appear more robust and equilateral in shape (Griesemer et al., 1963). The alae are also significantly different in the adult *Toxocara canis* based on the figures of Höppli (1925) and those of the adult *Toxocara cati* by Glaue (1910a). The major difference is that the V-shaped electron-dense area (the "Flügelleiste" of these authors) of the adult extends internally from the periphery of the ala only about halfway toward its base.

The hypodermis of the infective-stage larva was similar to that described by Nichols (1956a, b) for the larvae of *Toxocara canis* and *Ascaris lumbricoides*. It was also found to be quite similar to the hypodermis of the third-stage larvae of *Toxascaris leonina, Baylisascaris procyonis, Hexametra leidyi,* and *Lagochilascaris sprenti,* as described by Bowman (1987). Allgen (1943a, b) described the morphology of the hypodermal cords of larval *Toxascaris leonina* and found that it was composed of single cells and that the synctium did not form until the worms reached sexual maturity. This was not found to be the case with larval *T. canis,* nor was it found to be the case in larval *Toxascaris leonina* that had been recovered from mice (Bowman, 1987).

The morphology of the lateral cords of the larval *Toxocara canis* are quite similar to those found in the adult worm. Höppli (1925) described that of the adult *T. canis* and found a morphology similar to that of the larvae. Glaue (1910a, b) and Martini (1909) described the morphology of the hypodermis of *Toxocara cati*. They found that there were 2 types of nuclei present in the lateral cords, 1 type in the medial portion and the other in the sublateral portions. Although the basic morphology is the same, no nuclei were noted in the present study in the medial line of the lateral cords except in areas posterior to the anus. Martini found very few nuclei in the lateral cords of specimens that were less than 6 cm long with the occasional nucleus that was found in the sublateral portions of the cord. It would thus appear that the nuclei present in larger adults develop after the worm has begun its growth as an adult.

Hinz (1962) described the ultrastructure of the hypodermis of the adults of *Parascaris equorum* whereas Bogoyavlenskii (1973) described the structure of the hypodermis of this and several other adult ascaridoids, including *Ascaris suum* and *Toxascaris leonina*. Bogoyavlenskii divided the hypodermis of the adult into 3 layers based on the appearance of the fibrils contained therein. The layer closest to the cuticle, one-fifth the total thickness of the hypodermis, contained large numbers of annularly arranged fibrils. The widest zone, being more than one-half the entire width of the hypodermis, was the middle zone, which contained large numbers of vacuoles and a branched, plexus organization of fibrils. The area adjacent to the musculature was found to contain large numbers of annular and longitudinal fibrils. Hinz noted that the hypodermis was rich in endoplasmic reticulum and that the nuclei were concentrated around the nervous tissue of the ventral nerve cord. There was no attempt made to determine the various layers of the hypodermis in the study reported here.

The ultrastructure of the muscle cells of the

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Figures 22–25. Electron micrographs of the infective-stage larva of *Toxocara canis*. Bars = 1 μm for Figures 22–24 and 2 μm for Figure 25. 22. Transverse section posterior to the rectum. Note the inward expansion of the lateral cords and the presence of nuclei within the pseudocoelom. 23. Transverse section near the tip of the tail. Note the large ventral cord containing lipid granules that fill most of the pseudocoelom. 24. Longitudinal section through the tail. Note the rectum, the large number of nuclei encircling the rectal canal, and the numerous nuclei posterior to the rectum. 25. Longitudinal section through an intestinal cell. The large nucleus of this cell can be noted at one end of the cell.

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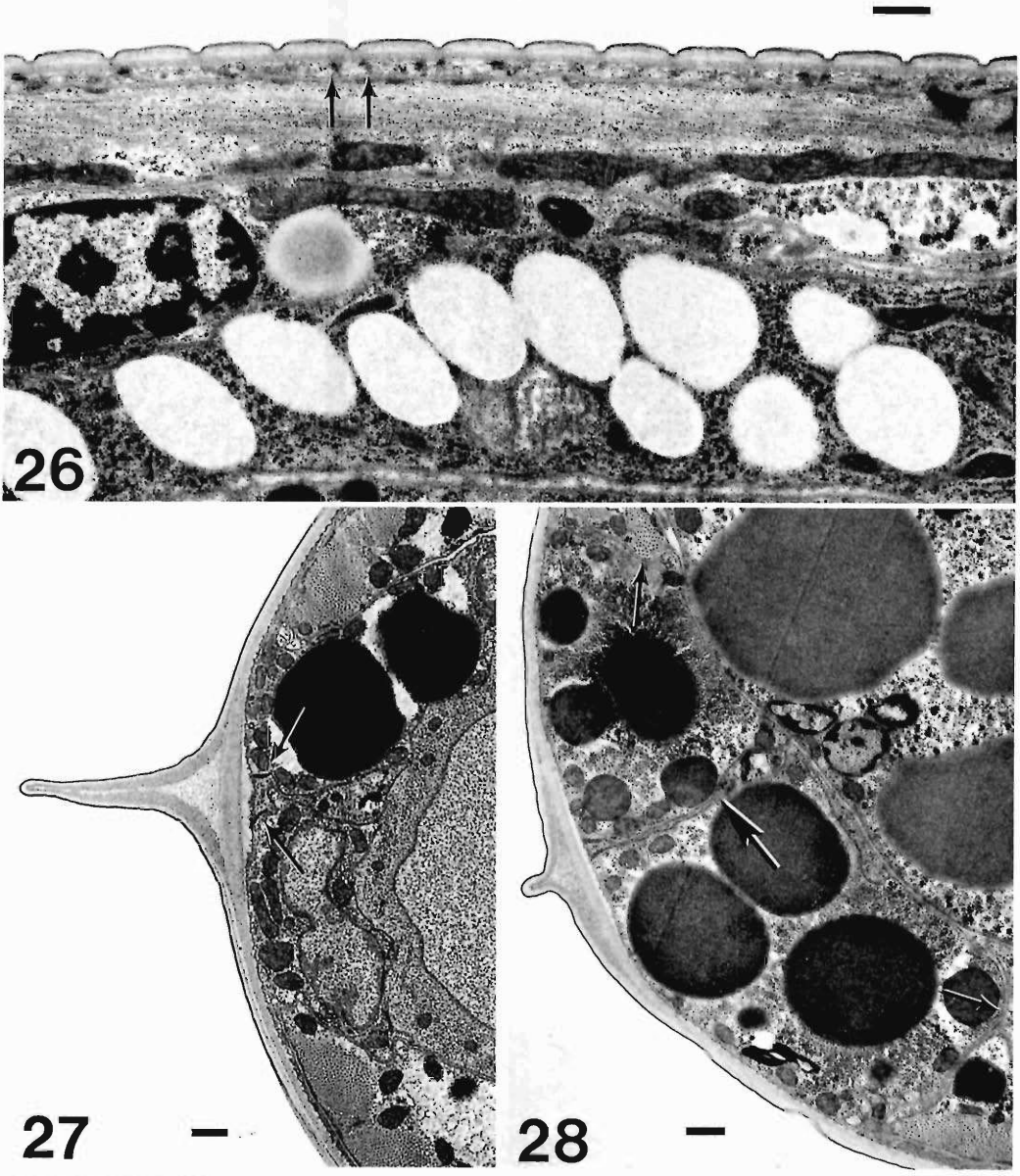

Figures 26–28. Electron micrographs of the infective-stage larva of *Toxocara canis*. Bars = 0.5 μm. 26. Enlargement of the cuticle on the dorsal surface (Fig. 17) showing the various cuticular layers; thickenings at the base of each stria are formed from the fibrillar layer (arrows). 27. Enlargement of the left lateral ala (Fig. 12) showing its cuticular composition and desmosomes marking the connection with the medial portion of the lateral cord (arrows). 28. Enlargement of the left lateral cord (Fig. 20) showing the medial (large arrow) and sublateral portions of the cord (dorsal and ventral extent marked with a small arrow).

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infective-stage larva of *T. canis* revealed that there were few myocytes per quadrant and that these cells were of a meromyarian and platymyarian type. This differs from adult ascaridoids, wherein the merocyte organization is polymyarian and coelomyarian (Wright, 1966). Nichols (1956a) did not distinguish individual muscle cells in the infective-stage larvae of *T. canis*. Stretton (1976), however, noted that the infective-stage larva of *Ascaris suum* had a total of 83 stomatic muscle cells while the adult had a total of about 50,000 such cells. Thus, it would appear that an increase in myocyte number along with an accompanying change in myocyte morphology may be a common phenomenon within polymyarian ascaridoidea.

The muscle bridges of *Toxocara canis* adults were examined by Wright (1966), who showed that cytoplasmic portions of individual muscle cells often connected to both the ventral and lateral cords. They were found to be much like the muscle cells of the adults of *Ascaris lumbricoides* and *A. suum*, which have received considerable attention since Schneider (1866) noted that they differed from the muscles of other animals in that the cytoplasmic portions of the muscle fibers extend from the muscles to the nerves in the ventral or dorsal nerve cords (for review, see DeBell, 1965; also, Stretton, 1976).

Work by Bartnik et al. (1986) and Francis and Waterston (1991) has shown that the somatic musculature of nematodes is attached to the cuticle by desmosome-linked tonofilaments that are immunochemically similar to intermediate filaments of mammals. These filaments are believed to cross the hypodermal cell and act as a means of attachment between the muscle cell and the cuticle. The presence of tonofilaments in the hypodermis of the larva of *T. canis* suggests that the mode of muscle attachment to the cuticle is similar in this larval nematode.

The nervous system of the infective-stage larva of *T. canis* was much like that described by Nichols (1956a). Nichols, however, was not able to distinguish the dorsal, ventral, and lateral cords, although he did note that prominent nuclei could be observed in the ventral line. The nervous system of the adult of *A. suum* was extensively mapped by Goldschmidt (1903, 1908, 1909, 1910) and more recently by Stretton et al. (1978) and has been shown to consist of about 250 neurons, of which there are 162 in the nerve ring and anterior ganglia. As of this time, the number of nerve cells in the larva has not been counted, but it is believed that the number is very similar to that of the adult worm (Stretton, 1976).

The ultrastructure of the excretory cell described here for the larva of *T. canis* is similar to that described by Kondo et al. (1987) and by Vegni-Talluri and Dallai (1990). The ultrastructure of the excretory cell of the *T. canis* larva is also very similar to what has been described for other larval ascaridoids, i.e., the infective-stage larva of *Ascaris suum* (Jenkins, 1971), larval *Anisakis* (Lee et al., 1973), and larval *Phocanema* (*Pseudoterranova*) *decipiens* (Davey and Sommerville, 1974). The most striking feature of these cells is the large nucleus, the large numbers of membrane-bound vesicles, and the presence of numerous mitochondria, Golgi bodies, and RER, all features of a metabolically active secretory cell. Davey and Sommerville (1974) postulated that enzymes in the cell remain dormant until the cell is stimulated by neurosecretory cells at the time of molting. It would appear that this may be one function of the cell, although other functions have been suggested, including osmoregulation (Beherenz, 1956), exodigestion (Mueller, 1929; Lee, 1970), acetylcholine inhibition (Ogilvie and Jones, 1971), and substrate secretion to assist in motility (Bird, 1990). In culture, *T. canis* larvae produce large amounts of protein (Badley et al., 1987a), and antibodies to these proteins bind strongly to the excretory cells of larval *T. canis* in histological sections (James Parsons, unpubl. obs.). These data suggest that the excretory cell of the larval *T. canis* is actively producing proteins during this phase of the life cycle that is usually found within a paratenic host. Robertson et al. (1989) showed that proteases are a component of the excretory–secretory antigens produced by cultured larval *T. canis*. This enzymatic activity and the immunolocalization of these antigens to the excretory cell suggests that this cell is involved in the production and exportation of proteolytic enzymes to the external environment of the worm. For these reasons, it has been suggested that the cell should be termed a secretory cell (Maizels and Page, pers. comm.). However, it may be that the proteolytic portion of the excretory–secretory product is actually being produced by the esophageal gland cells, which, as already described, are known to produce proteases. In fact, some monoclonal antibodies to excretory–secretory antigens bind to the secretory cell, whereas others bind to

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the esophageal region; thus, the origin of the proteases may be either, or both, sources (Maizels and Page, pers. comm.). Certain monoclonal antibodies that are reactive with the excretory–secretory products are also reactive with the larval surface (Bowman et al., 1987), and component(s) of polyclonal antibody that mediate cellular attachment to larvae in vitro can be removed by preabsorption of the antibody with excretory–secretory product (Badley et al., 1987b). This sharing of antigenic epitopes by the surface of the larvae and the excretory cell suggest the possibility of the export of excretory–secretory proteins to the larval surface; however, the mechanisms underlying transport of these antigens to the larval surface from the excretory cell has not been elucidated.

The general ultrastructural morphology of the excretory cell is similar to that reported for other nematodes. The work of Narang (1970, 1972) with *Enoplus brevis, Pangrellus redivivus, Ditylenchus* spp., and *Heterodera rostochiensis,* Waddell (1968) with *Stephanurus dentatus,* Lee (1970) with *Nippostrongylus brasiliensis,* Nelson et al. (1983) with *Caenorhabditis elegans,* and Endo and Wergin (1988) with *Meloidogyne incognita* all show a similar pattern of morphological organization. This pattern consists of a single large cell that contains a large nucleus, large quantities of endoplasmic reticulum, membrane-bound vesicles, and canaliculi lined with a thickened cuticlelike material. Associated with this cell are several supporting cells that number only 3 in the case of *C. elegans* but are possibly more numerous in other nematode species.

Acknowledgments

The authors thank Mrs. Renate Bromberg for her excellent assistance with the electron microscopy. They also thank Dr. M. D. Little for his critical comments on the manuscript prior to its submission. This work was sponsored in part by NIH grant EY05677.

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